

TISSUE WELDING WITH 980-nm DIODE LASER SYSTEM

by

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For what good science tries to eliminate, good art seeks to provoke -mystery, which is lethal to the one, and vital to the other.

John Fowles

ABSTRACT

TISSUE WELDING WITH 980-nm DIODE LASER SYSTEM

In this study, 980 nm diode laser system is used as a novel tool for tissue welding. To our knowledge, this is the first study on the effects of 980-nm laser on tissue welding. Hence, a preliminary study was done to determine optimal parameters for further studies. 1 cm long incisions, which were created on the Wistar rat's dorsal skin were welded. Tissue welding with 980-nm wavelength depends on the degree of photothermal interaction. Thus, different power levels and exposure schedule were investigated. By using estimated optimal parameters, a second study was performed. In the second study, three of the 1 cm long incisions, which were created on the dorsal skin of each Wistar rat were welded with laser and the other three incisions were sutured. Wounds were biopsied at 1,4,7,14 and 21 days postoperatively. Histological measurements and statistical analysis showed that there were no statistically significant differences in the epidermal thickness and granulation of wounds that were closed either by laser welding or sutures. Dorsal sides of all animals were photographed from the date of surgery until the animals were sacrificed. The clinical examination – opening of wound and checking for presence of infection – was performed. Wounds were welded successfully at the end of the study. Laser welded and sutured wounds showed similar healing patterns. Thus, 980-nm diode laser was said to be a good candidate for tissue welding applications.

Keywords: 980-nm diode laser, laser tissue welding, wound healing, epidermal thickness, granulation.

ÖZET

980-nm DİYOT LASER İLE DOKU KAYNAĞI

Bu çalışmada, 980 nm diyot laser sistemi, doku kaynağı yapmak için yeni bir yöntem olarak kullanılmıştır. Bu çalışma, 980-nm diyot laserin doku kaynağı üzerindeki etkilerinin araştırılması açısından bir ilki oluşturmaktadır. Bu nedenle, optimum laser parametrelerinin belirlenmesine yönelik bir ön çalışmanın yapılması gerekli görülmüştür. Wistar cinsi sıçanların sırt bölge derisinde açılan 1 cm uzunluğundaki yaralara, 980 nm dalga boylu laser kullanılarak kaynak yapılmıştır. 980-nm dalga boyunda yapılan doku kaynağının başarısı fototermal etkileşimlerin miktarına bağlılık gösterir. Bu nedenle çeşitli güç seviyeleri ve laser uygulama zaman dizgileri denenmiştir. Bu ön çalışma sonucunda belirlenen optimum parametreler kullanılarak ikinci çalışma gerçekleştirilmiştir. İkinci çalışmada, her bir Wistar cinsi sıçanının sırt bölgesinde açılan 1 cm uzunluğundaki yaralardan 3 tanesi laser kullanılarak doku kaynağı ile kapatılmış, diğer üç yara ise klasik dikiş yöntemiyle kapatılmıştır. Yaralar operasyonların ardından 1.,4.,7.,14. ve 21. günlerde biyopsiye tabii tutulmuştur. Histolojik ölçümler ve istatistik analizler sonucunda laser ya da dikiş ile kapatılan yaralarda epidermal kalınlık, granulasyon gibi faktörlerin istatistiksel olarak anlamlı farklılıklar göstermediği belirlenmiştir. Tüm deney hayvanlarının sırt bölgelerinin operasyon gününden, hayvanların itlaf edildiği tarihe kadar fotoğrafları çekilmiştir. Yaranın durumu (enfeksiyon, açılma vb) kaydedilmiştir. Çalışmanın sonucunda, yaraların doku kaynağı yöntemi ile başarılı bir şekilde kapatıldığı saptanmıştır. Laser kaynağı ve dikişle kapanan yaraların benzer iyileşme seyri izledikleri gözlemlenmiştir. Buna dayanarak, 980-nm diyot laser sisteminin doku kaynağı uygulamasında kullanılabilir bir araç olduğu yargısına varılmıştır.

Anahtar Sözcükler: 980-nm diyot laser, laser doku kaynağı, yara iyileşmesi, epidermis kalınlığı, granulasyon..

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LIST OF ABBREVIATIONS

<i>A</i>	Amper
<i>C</i>	Centigrade
<i>CO₂</i>	Carbondioxide
<i>cm</i>	Centimeter
<i>fs</i>	Femtosecond
<i>h</i>	Hour
H&E	Hematoxylin and Eosin
Ho:YAG	Holmium Yttrium Aluminum Garnet
<i>J</i>	Joule
<i>IR</i>	Infrared
<i>kg</i>	Kilogram
<i>LASER</i>	Light Amplification by Stimulated of Radiation
<i>mm</i>	Milimeter
<i>ml</i>	Mililiter
μ	Micro
μm	Micrometer
<i>nm</i>	Nanometer
Nd-YAG	Neodymium Yttrium Aluminum Garnet
Nd-YLF	Neodymium Yttrium Lithium Fluoride
PDT	Photodynamic Therapy
<i>ps</i>	Picosecond
<i>TFC</i>	Temperature Feedback Control
<i>Ti-Sapphire</i>	Titanium Sapphire
UV	Ultraviolet
<i>W</i>	Watt

1. INTRODUCTION

1.1 Motivation and Objectives

Currently, lasers find wide application areas in clinical sciences. Laser welding is one of the most promising examples of the laser applications in medicine. It is a potential alternative technique for closing of tissues, which is getting transformed from the laboratory to clinical practice. Increased immediate wound strength, fluid-tight closure, faster operative repair, reduced susceptibility to any foreign body reaction, being non-lithogenic, improved cosmetic results (minimal scar formation) are the major advantages of tissue welding over conventional suture techniques.

There are lots of parameters that need to be optimized in tissue welding technique. Both laser and tissue properties must be considered to determine the optimum operation parameters. Wavelength, spot size, exposure time, power, mode of operation (pulse or continuous wave (CW)), number of pulse cycles and on/off durations of laser source and tissue optical characteristics (absorption, scattering and transmission) play major role on the efficacy of the fusion process.

The aim of this study was to examine the effects of 980-nm diode laser on skin welding. For that purpose, preliminary studies were done on Wistar rats to determine the optimum parameters for skin closure. According to the results of histological examinations, the optimum parameters of laser were investigated. The effects of 980-nm diode laser with those parameters and suture technique were analyzed and compared with clinical and histological examinations (Hematoxyline & Eosin).

1.2 Outline

In Chapter 2, medical use of lasers, laser tissue interactions, lasers in tissue welding, skin physiology and functions, and wound healing are explained.

Chapter 3 gives detailed information about methods used in this study such as tissue welding with 980-nm diode laser, experimental procedure, and histological evaluation.

Results about thermal effects of clinical examinations of preliminary studies, histological evaluation of preliminary studies, laser tissue welding and suture technique are discussed in Chapter 4.

In Chapter 5, a general discussion and limitations of this study can be found.

Chapter 6 covers conclusion and contribution of this study for further scientific works.

2. BACKGROUND

2.1 Laser in Clinical Realm

There has been interesting developments in technologies to amplify light, after Albert Einstein developed his quantum theory principles. Field of Quantum mechanics had emerged in 1917, but the first success in amplifying light came in 1954 by Gordon et al. when they created maser which is an acronym for Microwave Amplifier by Stimulated Emission of Radiation. Schwalow and Townes developed amplifier for visible light. Maiman who constructed the first optical amplifier, successfully developed the laser (Light Amplification by Stimulated Emission of Radiation). The first successful operation of a laser device was performed by Maiman with ruby crystal in 1960 [1].

Nowadays, lasers are widely used in various clinical applications. In the clinical area lasers were first applied in ophthalmology. Ability of lasers to photocoagulate tissues bring about lasers continues to be used by ophthalmologists today. The second clinical discipline was the dentistry, where those lasers were introduced. Lasers were also used for their ablative effects. Photodynamic therapy (PDT) and laser-induced interstitial thermotherapy (LITT) are the other main clinical laser research areas [2].

Dermatology, orthopedics, cardiology, and gastroenterology are the other medical areas of lasers that successful laser treatments have been performed. It is expected that additional clinical applications will be developed in the near future [2]. Understanding laser-tissue interaction mechanisms is essential to find appropriate laser and operation parameters for surgical or medical applications.

2.2 Laser-Tissue Interaction Mechanisms

Generally, laser radiation affects tissues, which absorb the radiation. The absorption of laser radiation in tissues, especially in ocular tissue, is strongly wavelength dependent. The type of interaction depends on the wavelength, applied energy, focal spot size, energy density and interaction duration. Depending on the above parameters, one of the following laser tissue interactions may occur: *thermal interaction, photoablation, plasma-induced*

ablation, photodisruption, photochemical interaction, thermal interaction. Five basic interaction types are shown in double-logarithmic map in Figure 2.1 [3,4].

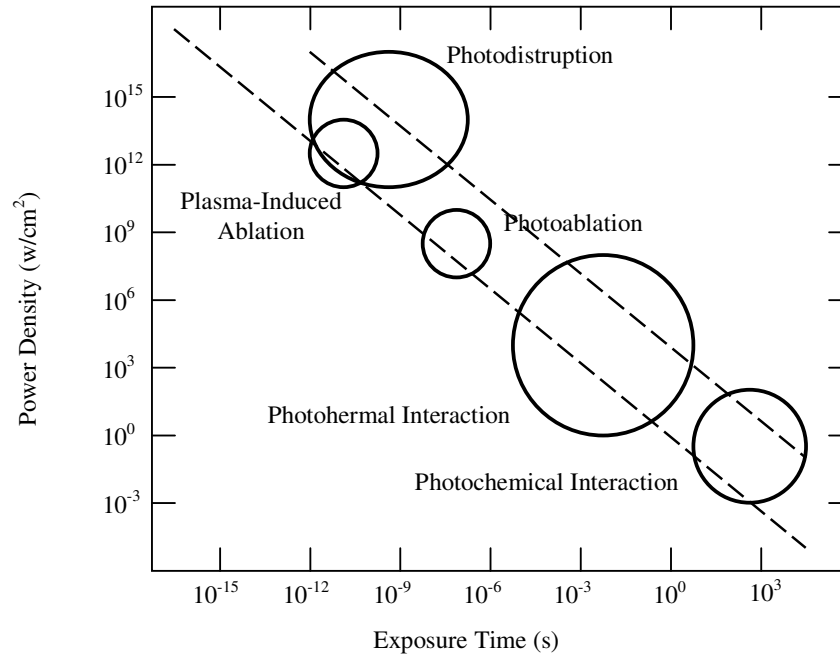


Figure 2.1 Representation of laser-tissue interactions. The circles give only a rough estimate of the associated laser parameters [2].

The interaction of laser light with tissue also depends on the optical properties of the tissue which is determined by the structure, water content, blood circulation, conductivity, heat capacity and density of tissue [2].

The effects of biological materials in the tissue vary significantly with the wavelength of the radiation. Water is a good example, which represents the main absorber in the infrared spectral region (Figure 2.2) [5].

Photochemical interactions stem from chemical reactions of light within macromolecules and tissues. Frequent applications of photochemical interaction are photodynamic therapy (PDT) and biostimulation. Photochemical interactions occur at very low power densities. Typical power densities range from 0.01 to 50 W/cm² and exposure times range from seconds to continuous wave [4].

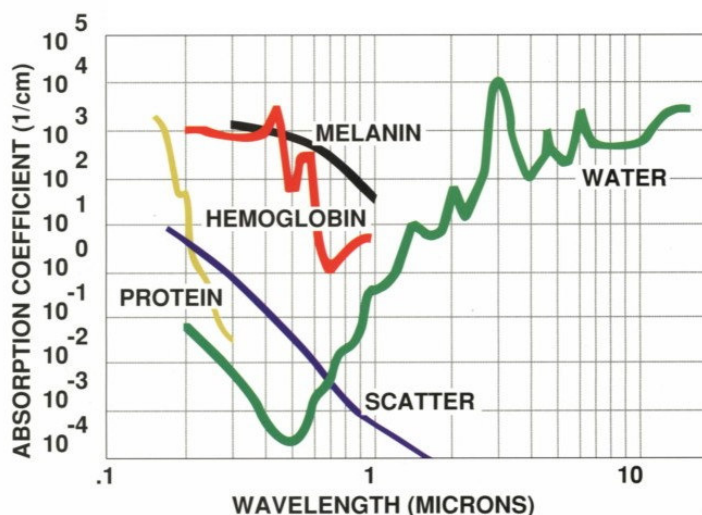


Figure 2.2 Spectral absorption of water and other biological materials [6].

In photoablation, the removal of tissue is performed without any appearance of thermal damage such as vaporization and coagulation. Power densities of photoablation are 10^7 - 10^8 W/cm² at laser pulse durations of nanosecond. Photoablation is one of the prosperous techniques for refractive corneal surgery. The refractive power of the cornea is altered in myopia, hyperopia, or astigmatism in refractive corneal surgery [3].

The other type of laser tissue interaction is plasma-induced ablation. The main idea is very clean removal of tissue without thermal or mechanical damage. Nd-YAG, Nd-YLF, Ti-Sapphire lasers are the typical lasers of this type of laser tissue interactions. 10^{11} to 10^{13} W/cm² power densities in a duration ranging from 100 fs to ps are applied in plasma-induced ablation. Plasma-induced ablation is also referred as plasma-mediated ablation. Local electric field strength which determines when optical breakdown is achieved is the key parameter. Optical breakdown occurs when the local electric field strength exceeds a certain threshold value, i.e. if the applied electric field forces the molecules and atoms to ionize. The term optical breakdown especially emphasizes that UV, visible, and IR light is strongly absorbed by the plasma [5].

Photodisruption has a very similar mechanism with the plasma-induced ablation. Photodisruption also induces optical breakdown in tissue for fragmentation and cutting of tissue for special applications such as lens fragmentation and lithotripsy. Nd-YAG, Nd:YLF, Ti:Sapphire with power densities between 10^{11} and 10^{16} W/cm² are the mostly used solid state lasers in photodisruption [5].

2.3 Photothermal Interactions

In clinical practice the effect of heat on tissue is the most widely encountered category of tissue interaction. Thermal interaction causes significant changes in tissue with the increase in local temperature. A variety of factors combines to determine the nature and extent of thermal effect: the energy or power density, exposure time, spot size and wavelength of light. Thermal interaction occurs when the power densities of CW radiation or pulse duration are between 10 and 10^6 W/cm^2 and ranging from $1\mu\text{s}$ to 1 minute. CO_2 , Nd:YAG, Er:YAG, Ho:YAG, argon ion and diode lasers are typical lasers for thermal interaction. Different effects like coagulation, vaporization, carbonization and melting may be distinguished depending on the duration and peak value of the tissue temperature (Figure 2.3) [4, 7].

Heat effects depend on the tissue type and local temperature. The first effect is conformational changes of molecules with bond destruction and membrane alterations, when the tissue is thermally affected. Between 42 °C and 50 , °C hyperthermia occurs. Above 50 and 60 °C, the enzymatic activities are reduced by denaturation of the proteins, collagens lipids and hemoglobin. This effect leads to coagulation of tissue and necrosis of cells.

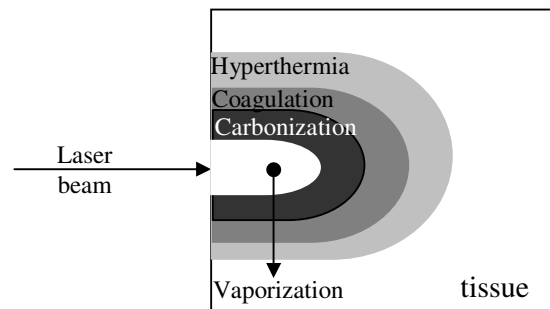


Figure 2.3 Localization of thermal effects in the biological tissue [2].

At higher temperatures exceeding 80 °C the breaking of the membranes becomes predominant in cells of all types. Vaporization begins at 100 °C; gas bubbles are formed inducing mechanical and thermal decomposition of tissue due to the large increase in volume during phase transition. Carbonization takes place which is observable by the

blackening of adjacent tissue and the escape of tissue above 100 °C. Melting can occur depending on the tissue after 300 °C [5].

Both the output of laser and the spot size of beam determine the power density and distribution on the tissue after light reaches the target size. The combination of laser wavelength and the absorption characteristics of different tissues determine three dimensional thermal response of the tissue, which will be induced. The optimal medical and surgical effects of cutting, coagulation, vaporization, or the delivery of homogenous low levels of radiation over a broad area can be obtained by means of manipulating above factors [8].

Photovaporization, which increases the quality of cutting and photocoagulation, which prevents bleeding are the two general applications of thermal interactions in surgical lasers. Time duration, magnitude and extend of heat deposited in the tissue determine the extent of tissue damage in photothermal applications. Conversion of laser energy to heat within applied tissue is predominantly governed by the dose rate and optical penetration depth of the laser beam. Tissue heating may not occur slowly due to insufficient amount of laser energy converted to heat, which is a result of slow energy delivery or minimal absorption. This situation causes the conduction of heat to the surrounding tissues and dissipation without any effect on tissue. An alternative outcome is a more generalized, but controlled, slow desiccation and photocoagulation of the target tissue. In coagulation, more laser energy can be absorbed and conducted as heat to surrounding tissue without vaporization since the gradual loss of tissue water leaves dehydrated tissue components that have a very high heat of vaporization [1]. The rate of heat delivery to the target tissue is increased by increasing power density or coefficient of tissue absorption. Rapid conversion of water to steam is caused by rapid energy delivery. At high temperatures, drastic changes in the absorption and scattering coefficients of the target tissue as well as the thermal conductivity and specific heat may be observed after phase transitions and water loss [1].

The application of heat to weld soft tissue is not new; laser has been used in tissue welding since 1965. Laser tissue welding undergoes modifications to suit the specific needs of this medical discipline. In spite of its advantage, several issues in laser tissue welding needs to be addressed before making transition to clinical use. These issues are

minimum thermal damage, best laser parameters for each application and exploring the types of tissues.

2.4 Lasers in Tissue Welding

Laser energy is used to anastomose tissues in lasers tissue welding system. In general, this energy results in some alteration of the molecular structure of the tissue being joined. The altered tissue molecules can form bonds of one sort or another with their neighbors. The introduction of protein solders and temperature-controlled feedback systems have led to acceptance of tissue welding in clinical medicine [9].

Suture closure is an effective technique, which has been used for centuries. Sutures are flexible, available, reliable, can be adapted to the most operative conditions and also cheap. However it is not a perfect technique. Seki found that suture has some defects like accuracy of placement and trauma to tissue from suture related forces. The position of needle in the holder, the slope of tissue at needle entrance and adjustments made during suturing to assure proper exit site, these technical factors affect suturing. Knot tension, choice of suture material and suture spacing also affect wound healing characteristics. Leaks can occur in the suture holes and at the space between sutures [3].

Suturing in other areas like endoscopy is currently not possible. In addition laparoscopic suturing is extremely difficult because of a two dimensional view, restricted instrument mobility and lack of direct manual contact.

Laser tissue welding is an improvement over conventional suture closure because it offers an immediate watertight tissue closure, decreased operative time (especially in laparoscopic and microsurgical applications), reduced trauma and elimination of foreign body reaction to sutures and clips. The main purpose of laser welding is to have an approximation that is durable, scarless, and resistant to infection.

Early experiments in tissue welding were performed by using electrocoagulation. Jain and Gorisch repaired incisions made in blood vessels of rats with Nd:Yag laser. This was the first successful example of laser welding. Jain showed that the Nd:Yag laser could be used successfully for microvascular anastomosis the following year [9]. Many

researchers have used different wavelengths lasers and settings to repair vascular anastomosis successfully. Most of the researches in laser tissue welding were about vascular anastomosis. However, tissue welding with laser system was also used to anastomose skin, nerve, biliary tissue, bowel and ureter [9].

In 1986, Studies of Abergel and then Garden followed the first early experiments. They concluded that the achievement of immediate sufficient tensile strength of the wound led to thermal damage and harmed wound healing. This observation was attributed to the important increase of temperature in tissue during welding, although crosslinking of collagen fibrils could be obtained only for temperatures over 60 °C. When this temperature was maintained for duration longer than 1 second, it led to an irreversible thermal damage and consequently to tissue coagulation and necrosis by burn injury. This damage delayed wound healing and resulted in increased scarring [10].

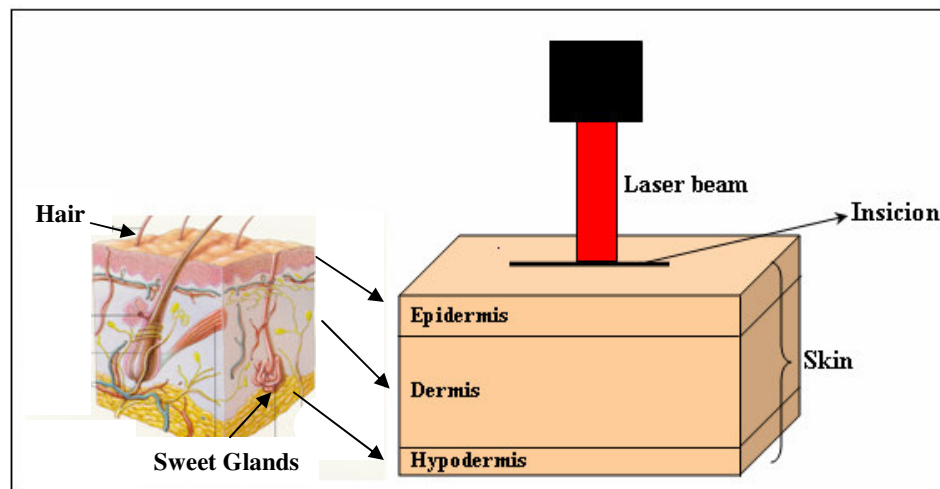


Figure 2.4 In the laser assisted skin closure group, the laser beam is applied through the incision. Revised from the Master Thesis of Özer [4].

To avoid these damages, several researchers have proposed adding dye or human albumin solders in the wound before processing. Thus, the energy of laser is specifically absorbed by the adjuvant. The heat is confined in the adjuvant instead of the tissue. So far, the results have been encouraging with immediate increased tensile strength, limited thermal damage and improved cosmetic aspect. Tissue welding is obtained by combination of a laser, a solder, and an exogenous growth factor, which stimulates the healing process.

Recent findings showed that early postoperative strength of laser-welded wounds could be increased by using this procedure. Although these results have been quite interesting, this technique seems to be demanding in daily surgical procedures and does not make the wound closure procedure easier [10].

Temperature feedback control of tissue welding was used in *in vitro* and *in vivo* models. It is found that laser-assisted anastomoses created in isolated segments of fresh canine jejunum *in vitro* were optimally strong at 90–95°C feedback control temperatures. Recently, this hypothesis was tested by welding rat small intestine segments *in vivo* with argon laser radiation under temperature feedback control (TFC) at 90°C control temperature and without temperature feedback [11].

In a comparative study done with 808 nm diode laser (0.95–1.05 W) with albumin soldering and Ho:YAG (2.01 μm) laser (0.24–1.13 W) without any soldering agent, the former showed better results than the latter on porcine posterior tibial arteries. The solder layer in 808-nm application protects the underlying tissue from direct laser irradiation and from desiccation during the heating process. In contrast, water acts as an absorbing chromophore in the Ho:YAG experiments where the tissue was welded without any additional solder. With increasing welding time, tissue desiccation led to a fast increase of the temperature visualized by the large number of vacuoles in the tissue and carbonization of tissue surface. This resulted in weakly welded tissue [12].

In the case of laser welding, some researchers have used lasers (Diode lasers or Nd:YAG), whose radiation penetrated deeply into tissue, whereas others have used lasers (e.g., CO₂) whose radiation is highly absorbed in the top surface layer. The “end point” was obtained by visual signs, such as changes in the color of the welded tissue. In the case of laser soldering, the researchers used various biological “solders,” such as albumin or fibrin that were heated by the lasers. These served as “biological scaffolding” that strengthened the welded zone. Blood was identified as an early solder, followed by egg-white albumin, and then other proteins, principally derived from blood: fibrinogen and other albumins [5, 13].

For CO₂ laser, optimal welding conditions are obtained for surface temperature $T = 60^\circ\text{C} = 333^\circ\text{K}$ and welding duration of $t = 12$ seconds. On the other hand, if the

temperature T is decreased by 15°C to $45^{\circ}\text{C} = 318^{\circ}\text{K}$, no welding will be observed. If the temperature is increased by 15°C to $75^{\circ}\text{C} = 348^{\circ}\text{K}$, there will be an excessive thermal damage that may lead to weak welding. Maximal strength was recorded when the temperature of the welded spot was 55°C and the exposure time was 12 seconds [14].

When using a Nd:YAG laser and a human albumin solder, weld temperature could affect bond strength in the viable range from 65°C to 95°C , with higher temperatures leading to stronger bonds, but temperatures above 95°C created extreme damage including ablation [13].

In an other study done with Nd:YAG laser (10 W), no solder was used. The total energy delivered to each incision was approximately 315 J, delivered in 75 scans. Each scan delivered 4.2 J of total energy to the weld site. To minimize thermal damage to the skin outside the weld area, the beam was blocked with high-reflecting metal plates placed on each end of the incision [15]. Lateral thermal damage in the laser welds was limited to $200 \pm 40\mu\text{m}$ near the epidermal surface with less thermal damage deeper within the dermis.

The mechanism of laser closure is not completely understood. This lack of basic understanding has been a major disadvantage for improvement of the technique. The difficulties in understanding the mechanism of laser welding arise from the multitude of lasers, parameters including laser wavelength, power output, exposure time and use of chromophores, and differences between tested tissues. These differences make it difficult to compare laser welding studies between each other [9].

Electromagnetic energy that applied in the form of laser light leads to the formation of tissue weld. The laser light is converted into heat in the tissue, and it is believed that this heat causes structural changes in the tissue.

In contravention of the difficulties above, several studies have attempted to define the mechanism. The first insight into underlying process involved in thermal tissue welding was provided by Schober et al. They found that tissue welding with $1.32\text{-}\mu\text{m}$ Nd-YAG laser in a rat carotid artery and sciatic nerve resulted in a homogenizing change

in the collagen with interdigitation of the individual fibers [3]. They reported that loosening of the collagen triple helix and some sort of interactions between collagen strands. They deduced that collagen “bonding” was responsible for laser welding.

The other study performed by White’s group found that argon laser welding of vein grafts in dogs led to direct collagen-to-collagen and collagen-to-elastin bonding. They correlated this finding with the surface temperature observed using infrared cameras and their argon laser welding technique. In their study maximum tissue temperature during laser welding with argon wavelengths was 48.8 °C, whereas it was significantly higher (84.0 °C) with the CO₂ laser [3]. The reason of these differences may be to get the same core temperature given the differences in tissue absorption between these two wavelengths.

Menovsky and colleagues found that the bonding mechanism for peripheral nerves was collagen-to-collagen attachment without interdigitation and individual collagen fibrils appeared homogenized, swollen, and fused. The other group, Tang et al, examined 830-nm diode laser aortorrhaphy repairs in rats by using electron microscopy and found that collagen fibers appeared fused, roped, swollen, or dissolved and were surrounded by normal collagen fibers. Tang concluded that cross-linking that occurs at the cut ends of the collagen fibers and the roping effect of parallel collagen fibers may be the underlying mechanism of welding [9].

In 1989, Murray et al reported results of an argon laser treatment and showed that both skin and arteries may either degrade or cross-link proteins. They noticed a decrease in the amount of a 235-kd guanidine-extractable protein from both guinea pig skin and canine blood vessels, which, they believed, could be fibronectin. They also found that the amount of type VI collagen was decreased and they found high-molecular-weight aggregates that were reducible by pepsin digestion, suggesting that aggregates were noncollagenous or had noncollagenous domains. They concluded that detectable biochemical alterations of the extracellular matrix occur and could be the result of covalent cross-links. They also suggests that these cross-links may not be solely related to collagen [9].

Helmsworth et al., used argon laser for extracellular matrices containing type IV collagen, laminin, entactin, and heparin sulfate proteoglycan. They found that the extracellular matrix proteins could be altered by the argon laser without covalent bond interactions and the welding effect is most likely the result of reorganization of the intramolecular disulfide bonds of laminin, type IV collagen, and entactin [3, 9].

Bass et al. performed welding with 808-nm diode laser by using indocyanine green dye. They found that covalent bonding was not responsible for the laser weld in rat type I collagen. Collagen was denatured, uncoiling the native triple helical structure. This work strongly suggests that welding occurs by some other noncovalent bonding mechanism [3, 9].

The tissues and laser system selected for experimentation plays an important role on the specific mechanism of laser welding. Extracellular proteins have more effect than collagen alone; however, collagen probably plays a major role [9].

Laser welding is an invasive method of joining or bonding tissues by using laser energy. There are three types of laser welding: Laser welding, laser soldering and dye enhanced laser welding. In laser welding technique tissues are joined thermally. Actual mechanism of this technique is still unknown; but increase in temperature of tissues conduces loosening of the collagen triple helix and some sort of interaction between collagen strands. In second type - laser soldering - a biological solder is applied to the wound edges and only then laser energy is applied. Different biological substances (bovine serum albumin, human serum albumin) are used as solders [16]. Third type is dye enhanced laser welding, using a dye to facilitate selective delivery of laser energy by the target tissue [9]. Laser tissue welding is relatively easy to perform. A few stay sutures or fingers can be used to approximate the tissue edges.

2.5 Skin Physiology and Functions

The skin is a metabolically active organ with vital functions such as protection and homeostasis. The most important functions of the skin are:

- Protecting the body from the environment, particularly from the sun
- Preventing excessive water loss from the body
- Protecting the body from infection.
- Regulating body temperature
- Mechanical support
- Sensory organ for touch, heat, cold, socio-sexual and emotional sensations
- Excretes toxic substances with sweat [4,17].

The skin is made up of three layers: the epidermis, the dermis and the hypodermis.

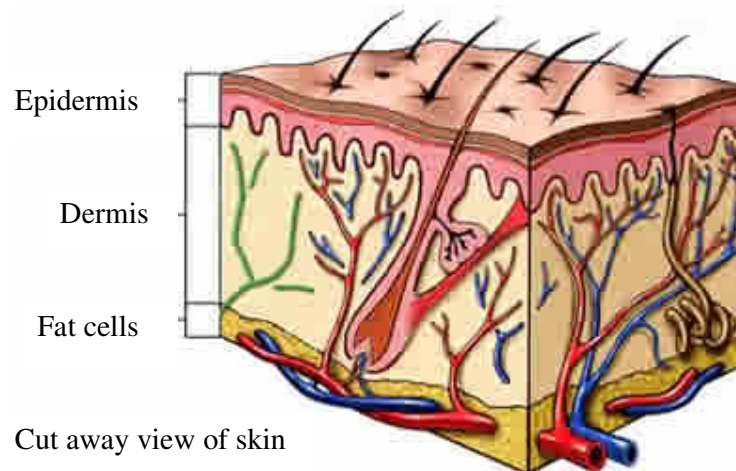


Figure 2.5 Skin Layers [18].

The epidermis is the outer layer of skin. The thickness of the epidermis varies in different types of skin. It is the thinnest on the eyelids at 0.05 mm and the thickest on the palms and soles at 1.5 mm. The epidermis contains 5 layers. From bottom to top the layers are named stratum basale, stratum spinosum, stratum granulosum, stratum lcidum, and stratum corneum. The bottom layer, the stratum basale, has cells that are shaped like columns. In this layer the cells divide and push already formed cells into higher layers. As the cells move into the higher layers, they flatten and eventually die. The top layer of the epidermis, the stratum corneum, is made of dead, flat skin cells that shed about every 2 weeks. There are three types of specialized cells in the epidermis. The melanocyte produces pigment (melanin), the Langerhans cell is the frontline defense of the immune system in the skin, and the Merkel's cell's function is not clearly known.

The dermis also varies in thickness depending on the location of the skin. It is 0.3 mm on the eyelid and 3 mm on the back. The dermis is composed of three types of tissue that are present throughout - not in layers. The types of tissue are collagen, elastic tissue, and reticular fibers.

The two layers of the dermis are the papillary and reticular layers. The upper, papillary layer contains a thin arrangement of collagen fibers. The lower, reticular layer is thicker and made of thick collagen fibers that are arranged parallel to the surface of the skin. The dermis contains many specialized cells and structures. The hair follicles are situated here with the erector pili muscle that attaches to each follicle. Sebaceous (oil) glands and apocrine (scent) glands are associated with the follicle. This layer also contains eccrine (sweat) glands, but they are not associated with hair follicles. Blood vessels and nerves course through this layer. The nerves transmit sensations of pain, itch, and temperature. There are also specialized nerve cells called Meissner's and Vater-Pacini corpuscles that transmit the sensations of touch and pressure.

The subcutaneous tissue is a layer of fat and connective tissue that houses larger blood vessels and nerves. This layer is important in the regulation of temperature of the skin itself and the body. The size of this layer varies throughout the body and from person to person [19].

2.6. Wound Healing

A wound is a body injury caused by physical means. Wound healing is the process by which injured part of the body is repaired. Twenty-one days is often regarded as the critical wound healing period. The process of wound repair differs little from one kind of tissue to another and is generally independent of the form of injury. Although the different elements of the wound healing process occur in a continuous, integrated manner, it is convenient to divide the overall process into three overlapping phases and several natural components for descriptive purposes [20].

There are three main phases in wound healing process: inflammation, proliferation and maturation. The sequential phases overlap and at each phase different cell types play key roles.

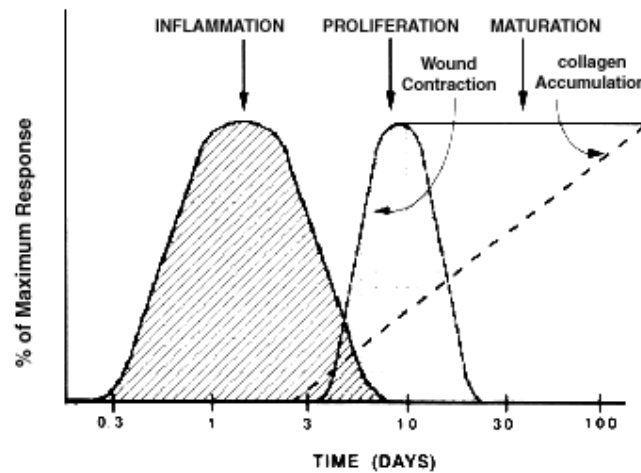


Figure.2.6 Phases of wound repair. Wound healing has been divided into three phases: inflammation, proliferation and maturation [21].

The healing response is initiated at the moment of injury. Surgical or traumatic wounds disrupt the tissue architecture and cause haemorrhage. Initially, blood fills the wound [20]. When platelets are released from damaged vessels exposure to the connective tissue and activation by interaction with collagen induces aggregation.

In inflammation, a number of neutrophil chemoattractants are generated at the site of injury. Their release induces a rapid increase in neutrophil numbers at the wound site within hours of injury. The role of neutrophils is restricted to destruction of bacteria contaminating the wound site. In the absence of wound infection the numbers of neutrophils rapidly decrease and they don't have a function in subsequent healing events.

At the same time as initiation of neutrophil chemotaxis monocytes and lymphocytes are attracted to the wound site. Monocytes differentiate into macrophages responding to the tissue environment. Within the immune response these cells phagocytose and kill bacteria. Macrophages also phagocytose cell debris, older neutrophils and dead tissue. Macrophages also play an important role in regulation of healing [22].

In proliferation phase of healing, tissue is formed. During the inflammatory phase many chemotactic and mitogenic factors are released and these initially attract fibroblasts and endothelial cells into the wound site and then stimulate them to proliferate [20]. Healthy granulation tissue appears red because of the presence of a high density of blood

vessels. It is a fragile tissue composed of a matrix of fibrin, fibronectin, proliferating endothelial cells and fibroblasts mixed with a population of inflammatory macrophages and lymphocytes.

Fibroblasts that migrate from the adjacent epidermis proliferate. They then undergo modulation to form myofibroblasts, which align into arrays parallel with the wound surface. The myofibroblasts has contractile feature and is thought to exert a cytoskeleton dependent tension on the granulation tissue and draw the wound edges together by contraction [4]. After wound closure the myofibroblast is removed by apoptosis and wound tension decreases.

Collagen is the major protein component of the extracellular matrix of skin. Wound extracellular matrix is composed of a mixture of collagen and elastin fibrils. The extracellular matrix plays a bioregulatory role in modifying the behavior of cells that come into contact with it and also by acting as a reservoir of bound growth factors and enzymes.

In maturation phase, remodeling and scarring occurs. Collagen is constantly being degraded and resynthesised even in normal intact skin. Following injury its rate of synthesis increases along with an increase in degradation. Collagen synthesis decreases to normal levels by day 21st after wounding. Remodelling of the collagen fibers by degradation and re-synthesis allows the wound to gain strength by re-orientation of collagen fibers. The resulting scar is less cellular than normal skin and never achieves the same tensile strength as uninjured skin [22]. Remodelling can continue for up to two years after injury as the healed wound becomes covered with mature tissue. The relative weakness of the scar compared to normal skin is a consequence of the collagen fiber bundle orientation and abnormal molecular cross linking. The fibers in normal skin are relatively randomly ordered whilst in scar tissue more of the fibers run in parallel. As well as poor cosmesis scar tissue tends to contract abnormally so that normal function can be lost where there are large areas of scarring such as following burn wounds [22].

3. METHOD

In this study, laser tissue welding was performed on the skin tissue. 1 cm long incisions, which were created on the Wistar rat's dorsal skin, were welded with laser welding and suture. Tissue welding with 980-nm wavelength depends on the degree of photothermal interaction. Thus different power levels and exposure schedule were investigated. Dorsal sides of all animals were photographed from the date of surgery until they were sacrificed. The clinical examination opening of wound and presence of infection was noted. According to the results of those experiments, 980-nm diode laser system were used to evaluate clinical, and histological examinations of tissue closure for comparing laser tissue welding with the suture technique.

3.1 Tissue Welding with 980-nm Diode Laser System: Preliminary Study for Determination of Optimal Parameters

3.1.1 980-nm Diode Laser System

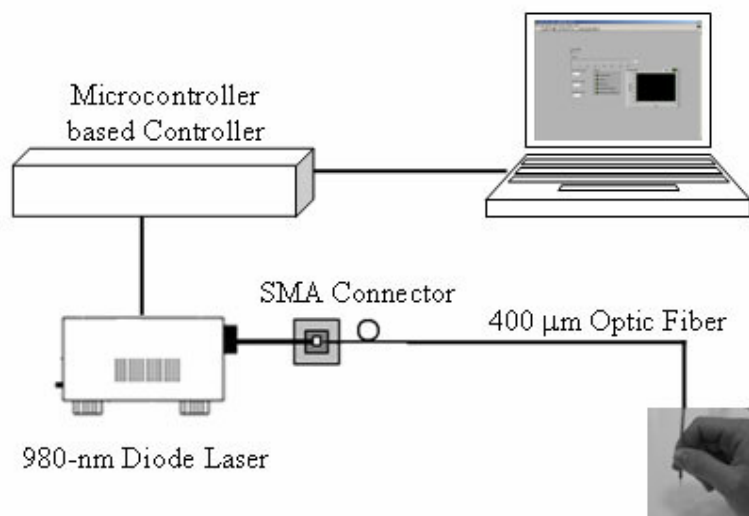


Figure 3.1 Laser Tissue Welding System.

The laser source (980-nm) was controlled by a controller which was designed and manufactured by the group at Biophotonics Laboratory at Institute of Biomedical

Engineering, Boğaziçi University. Welding was done with a fiber, fixed on a stage under computer control (Figure 3.1). 980-nm diode laser is available at Biophotonics Laboratory in Institute of Biomedical Engineering; Bogazici University (OPC-D010-980-FCPS, Opto Power Corporation, Tuscon, AZ, USA) with the connection between laser optical fiber output and 400- μ m silica coated optical fiber (Spindler–Hoyer, Göttingen, Germany). The parameters (power, exposure time, temperature and mode of operation (pulsed & CW)) of the lasers can be controlled by a user interface program (Labwiev 6.0) running on a PC.

3.1.2 Animals

A total of 3 male Wistar rats (age 5-6 months) from Psychobiology Laboratory of Boğaziçi University, weighing 240-250 grams, were used in the experiments. Subjects were housed in plastic cages. They were maintained on a 12 h light/12 h dark cycle (lights on at 07:00 am) in a temperature controlled vivarium (22 ± 2 °C). Food and water were available ad libitum.

3.1.3 Animal Preparation

1 cm long full-thickness incisions were made on the backs of rats. The experiments were performed on the dermal tissue of Wistar rat backs. Experiment animals were anesthetized with ketamin injection (1.65 ml/kg ketamidor 10%, RichterPharma, AG, Wels, Austria). The head parts of the animals were fixed with stereotaxy device in order to locate the back part of the animal properly. Hair on the back part of each animal was shaved using epilating cream and the dermal tissue is fully cleaned from hair. 6 wounds were formed on the back part of each animal using no. 11 scalpel. The wounds were formed in doubles that were parallel to the spinal cord, such that one wound was located to the right of the spinal cord and the other was located to the left (Figure 2).

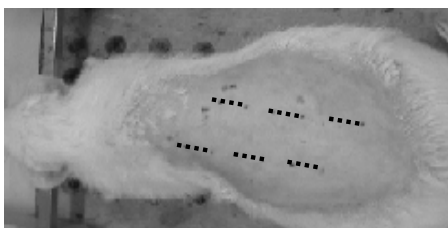


Figure 3.2 1-cm-long incisions on the dorsal side of the rat.

3.1.4 Experimental Procedure

Laser applications were performed as spots on skin using 400- μm optical fiber and laser, whose power, duration and mode was preadjusted. The optical fiber was located 2 mm above the tissue during all applications. The spot area has a diameter of 2 mm at this fiber-tissue distance.

3.1.5 Estimation of optimal laser parameters

A preliminary study of dose determination was performed for the 980-nm laser system in order to find the optimal parameters that minimize the thermal damage on neighboring tissues.

The preliminary study consists of two major experiments. In the first experiment, 6 wounds, each 1 cm long were formed on a single rat. Afterwards, these wounds were closed using 980-nm laser with varying parameters (Table I). In the second experiment, 6 wounds, each 1 cm long were formed on two rats (i.e. 12 wounds total). Then, these wounds were closed using 980-nm diode laser with different parameters, which were determined according to the results of the first experiment (Table II and III).

Table 3.1

980-nm Diode Laser Parameters Applied to the First Subject.

Wounds	Power (W)	Mode		Cycle Number	Energy (J)
		On (ms)	Off (ms)		
1	5	500	500	60	150
2	6	500	500	60	180
3	6	200	800	40	48
4	5	1000	1000	10	50
5	7	500	500	20	70
6	5	500	500	60	150

The total energy, E (J), incident on the surface of the 1-cm-length weld is given by the formula,

$$E = p \times t \quad (3.1)$$

where p is power (W) and t is time in seconds and is given as,

$$t = \text{Mode on (ms)} \times \text{cycle of number.}$$

In the first experiment, it was not possible to apply laser spot by spot since the optical fiber located above the wounds could not be adjusted to a fixed distance. Hence, energies per area was not calculated, and only the total energies were noted.

Table 3.2

980-nm Diode Laser Parameters Applied to The Second Subject.

Wounds	Power (W)	Mode		Cycle Number	Energy (J/mm ²)
		On (ms)	Off (ms)		
1-3	6	400	600	6	0.76
4-6	5	250	250	20	1.99

Table 3.3

980-nm Diode Laser Parameters Applied to The Third Subject.

Wounds	Power (W)	Mode		Cycle Number	Energy (J/mm ²)
		On (ms)	Off (ms)		
1-3	7.9	250	250	12	1.26
4-6	10	250	750	12	1.59

In the second experiment, the optical fiber was located 2 mm above the tissues during the laser applications. The spot area had a diameter of 2 mm. The energy per area was calculated using the formula,

$$E (J) = (p \times t) / \pi.r^2 \quad (3.2)$$

where r is diameter of spot area.

The antibiotic, Thiocilline (Abdi Ibrahim Ilac San. ve Tic. A.S. Istanbul-Turkey) was applied to the closed wounds immediately after laser tissue welding process to prevent infection and desiccation of the wounds.

3.1.6 Clinical Examinations

The wounds on the animals were examined and photographed daily after the welding experiments. Presence of wound reopening, infection, scar and hair formation was checked on a daily basis.

The animals were exterminated with injection of excess dose (4.95 ml/kg) ketamidor, 9 days after laser application to the animal of the first experiment and sixth day after laser application to the animals of the second experiment. Wound models were cut out with dimensions of 1 x 2 cm and fixed in formalin. The fixed wound models were preserved for further histopathology analysis.

3.2 Tissue Welding with 980-nm Diode Laser System: Main Study

3.2.1. Animals

A total of 10 male Wistar rats (at 5-6 months age and weighing 240-250 grams) from Psychobiology Laboratory of Boğaziçi University, were used in the experiments. Experimental procedure is the same as the part 3.1.3.

3.2.2 Welding Parameters

Three of six wounds in each 10 rats were closed with suture and the other three wounds were sealed with 980-nm laser system. For the wounds that served as controls, one full-thickness, simple interrupted Usp-3/0 metric- 275cm, silk sutures, (Doğsan Tibbi Malzeme San. A.Ş. Trabzon-Turkey) were placed on the 1 cm long incision.

Laser welding was performed with a pulsed mode, diode laser emitting radiation at a wavelength of 980-nm., 1 cycle 6W (22A), 400 ms on, 600ms off on per spot. There were 6 spots along 1cm incision. The total energy on the surface of the 1-cm-length weld is 14.4 J; the fluence at any particular point on one spot was 0.76 J / mm².

The antibiotic ointment was applied to the closed wounds immediately after laser

tissue welding process.

3.2.3 Histologic Evaluation

Dorsal sides of all animals were photographed from the date of surgery until they were sacrificed. The clinical examination – opening of wound and presence of infection – was noted. Histological analysis was performed on wounds closed by either laser or sutures on 1st, 4th, 7th, 14th, and 21st days postoperatively. On the appropriate day, the animals were sacrificed with an intraperitoneal overdose of ketamidol. The dorsal skin, including epidermis and dermis, was excised with a scalpel and sectioned into 1x 2 cm rectangles. The samples were fixed in formalin and processed for histology.

Samples are processed by using standard histologic techniques and stained with hematoxyline and eosin dyes. After fixation, skin wounds were embedded in paraffin. 3µm-thick sections in every 2mm were taken throughout wound part.

H&E procedure [2] was as follows:

1. Deparaffinized sections are taken on slides and slides are waited in Xylene 3x5.
2. Dipped with absolute alcohol 3x10.
3. Washed with tap water.
4. Stained with Harris Hematoxylin for 3 minutes.
5. Washed with tap water.
6. Purpled with Lithium-Carbonate 10 dip.
7. Washed with tap water.
8. Dipped with alcohol.
9. Stained with alcoholic Eosin for 5 minutes.
10. Washed with alcohol 4x10 dip.
11. Washed with acetone 2x10 dip.
12. Dried in etuve for 3 to 5 minutes.
13. Dipped in Xylene and covered with Entellan.

4. RESULTS

In this study, our aim was to examine the effects of 980-nm diode laser on skin welding. For that purpose, initial preliminary studies were done on Wistar rats to determine the optimum parameters for skin closure. Based on the results of histological examinations, the optimum parameters of laser were chosen for second study. The effects of 980-nm diode laser with those parameters and suture technique were analyzed with clinical and compared to histological examinations (Hematoxyline & Eosin).

Laser tissue welding applications have been proven to be a successful tool for wound closure in both studies. In addition, no anomalies were observed in health conditions, dietary habits or behaviours of the laser applied animals, whose health was examined daily after the laser application. After the closure of the wounds, the healing of the wounds is observed.

4.1 Clinical examinations of Preliminary Studies

4.1.1 Experiment I

In the first experiment, different parameter sets were applied on each wound (Table 3.1). The microscopic examinations revealed that the application on the 3rd wound (48 J) yielded the most effective closure and healing when compared to other wounds (Figure 3.1). No thermal damage was observed in the 3rd wound after visual inspection. Laser application on 5th (70 J) wound yielded comparable closure and healing with respect to the 3rd wound. However, some thermal damage was observed on the lateral parts of the 5th wound after laser exposure. Application on the 4th (50 J) wound is also considered to be efficient in terms of closure and healing but a more significant thermal damage was observed compared to 3rd and 5th wounds. Applications on 1st (150 J), 2nd (180 J) and 6th (150 J) wounds yielded closure but the analysis of the wound healing has not been feasible due to the extensive thermal damage.

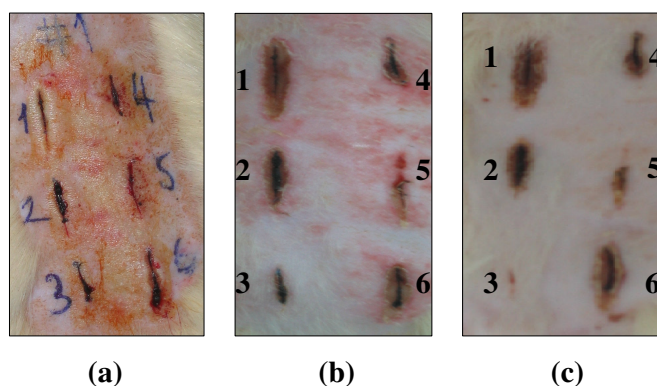


Figure 4.1 Photographs of the wounds of I. experiment at (a) 0th, (b) 5th and (c) 10th days. The Energies applied to wounds: 1-150J, 2-180J, 3-48J, 4-50J, 5-70J, and 6-150J.

4.1.2 Experiment II

Two animals were used in this part of the study. Identical parameters were applied during the tissue welding to each three of the six wounds on each animal. The parameters (Table 3.2, 3.3), which were decided based on the results of the Experiment I yielded promising results. No inconsistency is observed between the wounds, on which laser was applied with identical parameters.

Daily examinations revealed that, the wounds 1-3 (1.26 J/mm^2) on the second animal yielded the best macroscopic results (Figure 4.2 b, 4.3 b, 4.4 b). Effective tissue closure and healing was observed without any significant visual thermal damage. Wounds 4-6 (1.59 J/mm^2) on the second animal also yielded promising closure and healing without thermal damage (Figure 4.2 b, 4.3 b, 4.4 b). On the first animal, closure and healing was observed on wounds 1-3 (0.76 J/mm^2) again without any visual thermal damage. The wounds 4-6 (1.99 J/mm^2) of first animal yielded very similar results with wound number 4-6 and 1-3 of second animal.

The animals used in the experiment II are exterminated after the 6th day. A significant success of wound closure and healing is obtained. No major macroscopic variation is observed among the healing processes of the wound models in the experiment II.

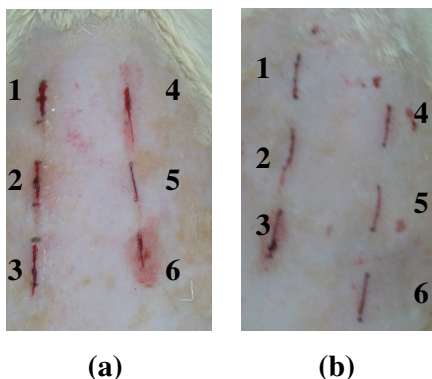


Figure 4.2 0th day of the II. experiment (a) 1th subject, (b) 2nd subject. The Energy applied to wounds :

(a) 1-3 = 0.76 J/mm² , (a) 4-6 = 1.99 J/mm²,(b) 1-3 = 1.26 J/mm², (b) 4-6 = 1.59 J/mm².

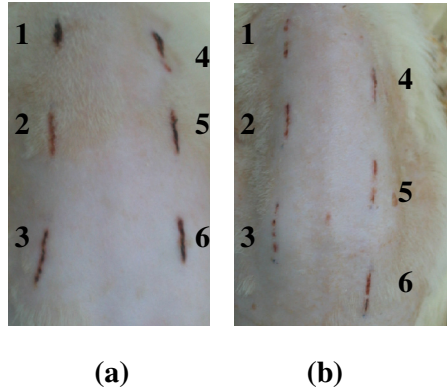


Figure 4.3 3th day of the II experiment (a) 1st subject , (b) 2nd subject. The Energy applied to wounds : (a) 1-3 = 0.76 J/mm² , (a) 4-6 = 1.99 J/mm² , (b) 1-3 = 1.26 J/mm², (b) 4-6 = 1.59 J/mm².

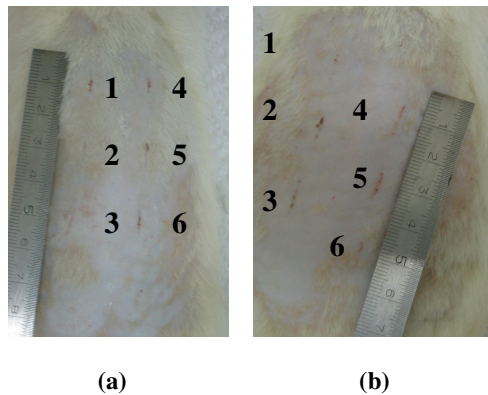


Figure 4.4 6th day of the II.experiment. (a) 1st subject, (b) 2nd subject. The Energy applied to wounds : (a) 1-3 = 0.76 J/mm² , (a) 4-6 = 1.99 J/mm² , (b) 1-3 = 1.26 J/mm² , (b) 4-6 = 1.59 J/mm².

4.2 Histological Evaluation of Preliminary Studies

Images of wounds taken at 6th days revealed a “funnel shaped” granulation zone. In the dermis, granulation tissue was filling the gap between the dermal wound edges with an average area of $\sim 165000\mu\text{m}^2$ and $218000\mu\text{m}^2$ in wounds (Table 4.1). The minimum granulation zone was observed in the wounds 1-3 (1.26 J/mm^2) on the second animal (Figure 4.5). The granulation zone of the wounds number 4-6 (1.59 J/mm^2) on the second

animal is more than the granulation zone of wounds 1-3 of second animal and the wounds 1-3 (0.79 J/mm²) & 4-6 (1.99 J/mm²) of the first animal.

Table 4.1

Average epidermal thickness and granulation of wounds.

Wound Numbers	Average Epidermal Thickness (μm)	Average Granulation (μm ²)
1-3 of first animal (0.76 J/mm ²)	85,48 ± 0,36	171117,5 ± 107826,4
4-6 of first animal (1.99 J/mm ²)	88,75 ± 9,51	179774,6 ± 46982,9
1-3 of second animal (1.26 J/mm ²)	71,89 ± 5,65	164952,6 ± 39602,74
4 -6 of second animal (1.59 J/mm ²)	78,84 ± 13,89	217424,7 ± 125644

Re-epithelialization was completed on the all wounds at day 6,. The width of epidermis is measured between ~ 71μm and 89μm on the wounds (Table 4.1). Wounds 1-3 of second animal yielded the minimum epidermal thickness. In the second animal, more epidermal thickness is observed on 4-6 wounds of second animal compared to 1-3 of second animal but 4-6 wounds of second animal has less epidermal thickness than 1-3 and 4-6 wounds of first animal (Figure 4.6). The average epidermal thickness of normal tissue that was not made incision and closed with laser or suture is ~ 45μm.

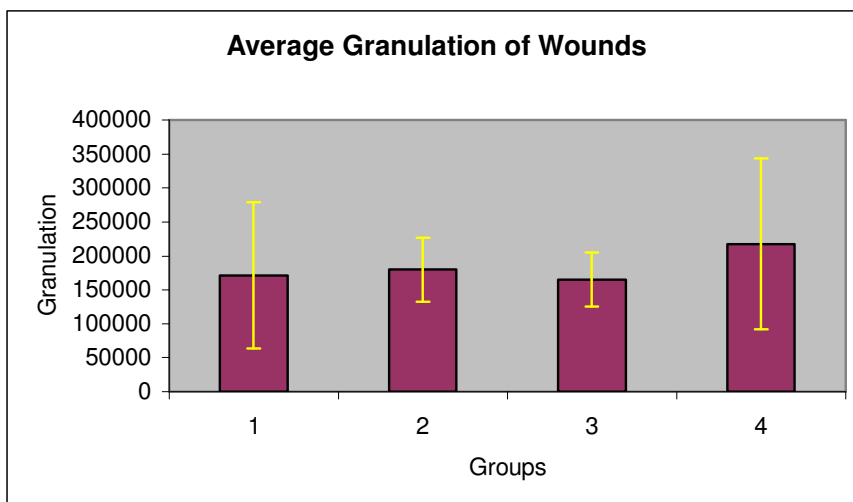


Figure 4.5 Average granulation tissue areas (μm^2) of wound groups. Groups; no.1 : 1-3 wounds of first animal, no.2 : 4-6 wounds of first animal, no.3: 1-3wounds of second animal, no.4 : 4-6 wounds of second animal.

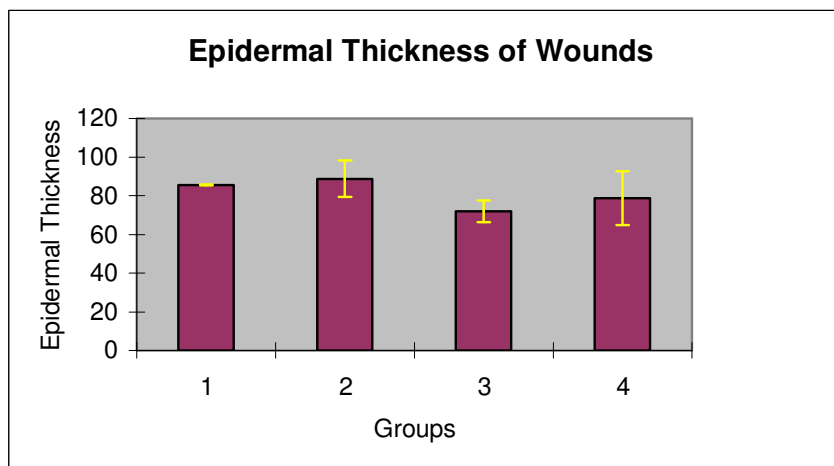
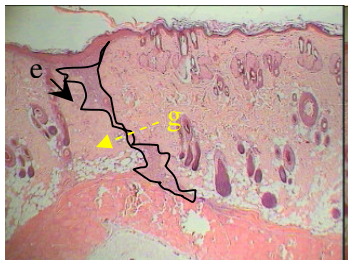
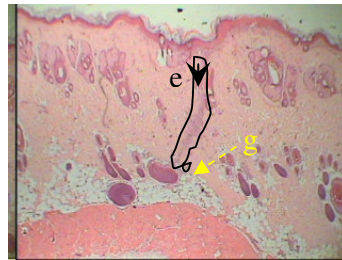


Figure 4.6 Average epidermal thickness (μm) of wounds. Groups; no.1: 1-3 wounds of first animal, no.2: 4-6 wounds of first animal, no.3: 1-3 wounds of second animal, no.4: 4-6 wounds of second animal.

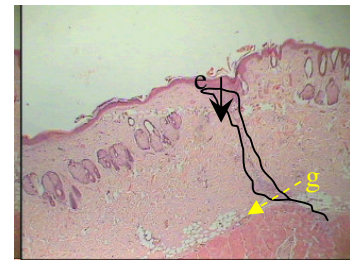
No foreign body reaction was observed in the wounds. In addition, no thermal damage evidence was noted in the tissues. No carbonaceous substances was seen over the epidermis.



1.wound of first animal

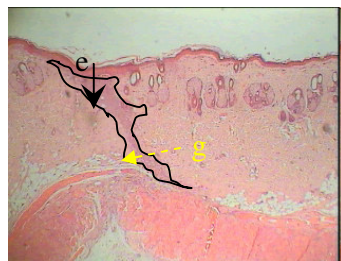


2.wound of first animal



3.wound of first animal

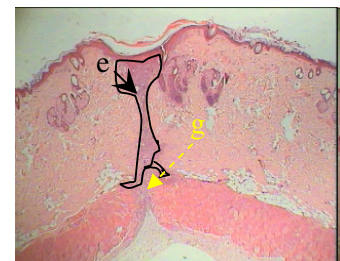
(The fluence at any particular point on one spot on the wounds was 0.76 J/mm^2)



4.wound of first animal



5.wound of first animal

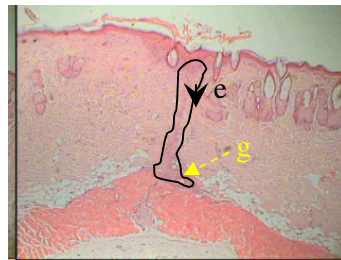


6.wound of first animal

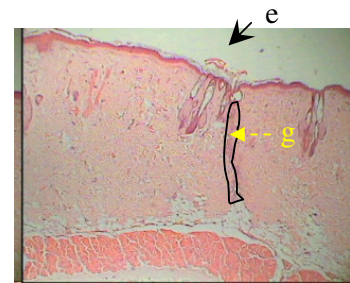
(The fluence at any particular point on one spot on the wounds was 1.99 J/mm^2)



1.wound of second animal

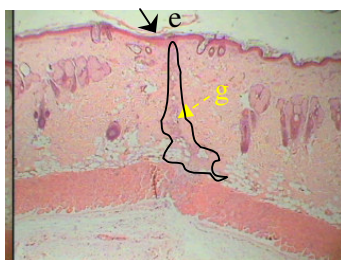


2.wound of second animal

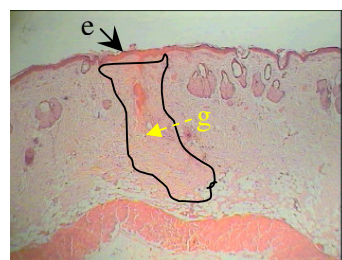


3.wound of second animal

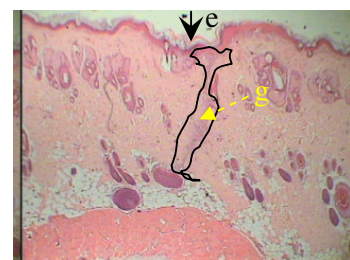
(The fluence at any particular point on one spot on the wounds was 1.26 J/mm^2)



4. wound of second animal



5.wound of second animal



6.wound of second animal

(The fluence at any particular point on one spot on the wounds was 1.99 J/mm^2)

Figure 4.7 Histological photographs of wounds (H&E,x4). Epidermal thickness' are shown by "e" arrows; granulation tissue areas are shown by "g" arrows.

4.3 Histological Evaluation of Sutured and Laser Welded Tissues

Routine stages of wound healing were observed on the wounds closed with sutures. Wounds remained open at the epidermis and below the epidermis 24 hours after the suture. Neutrophil leukocyte (Inflammatory cells) was already aggregating at the wound site. The epidermal thickness of wounds was $\sim 62.9\mu\text{m}$.

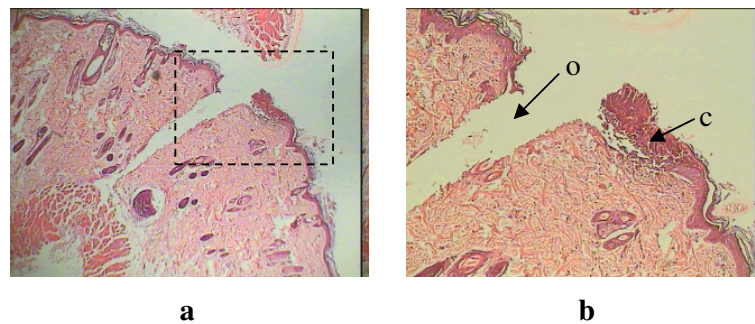


Figure 4.8 Healing of sutured tissue 24 hours after operation (H&E, a-x4, b-x10). Picture **b** is the x10 magnified part of the dashed rectangle of picture **a**. Clot is shown by “c”; “o” shows epidermis which was not anastomized.

By day 4, sutured wounds sites, where inflammatory cells were present, were closed at the epidermis, but remained open below epidermis. Migration of the epidermis beneath clot and epidermal hyperplasia was noted. The average epidermal thickness was $\sim 113.36\mu\text{m}$.

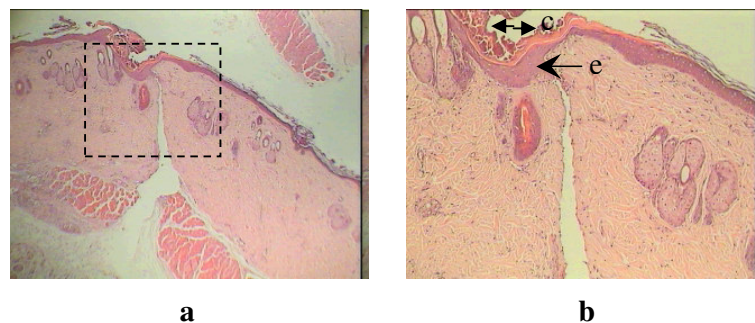


Figure 4.9 Healing of sutured tissue at day 4 (H&E, a-x4, b-x10). Picture **b** is the x10 magnified part of the dashed rectangle of picture **a**. Epidermis is shown by “e”; clot is shown by “c”.

By day 7, wounds were fully closed. A thin layer of granulation tissue that extended full-thickness through the skin was marked with deposition of fibroblast cells and collagen formation. The average epidermal thickness had decreased to $\sim 87.39\mu\text{m}$ and re-

epithelialization was completed. The granulation tissue area was $\sim 197190.3\mu\text{m}^2$ at the dermis. Inflammatory cells were no longer observed around the wound site by day 7.

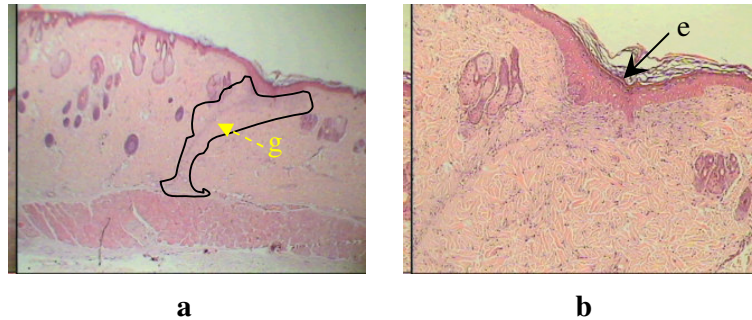


Figure 4.10 Healing of sutured tissue at day 7 (H&E, a-x4, b-x10). Picture **b** is the x10 magnified part of picture **a**. Epidermis is shown by “e”; granulation tissue area is shown by “g”.

At the 14th day, the epidermis was $\sim 62.04\mu\text{m}$ thick. Some of the tissue volume was replaced with granulation tissue while the epidermal hyperplasia began to decrease. The average granulation tissue area was $\sim 259115.2\mu\text{m}^2$.

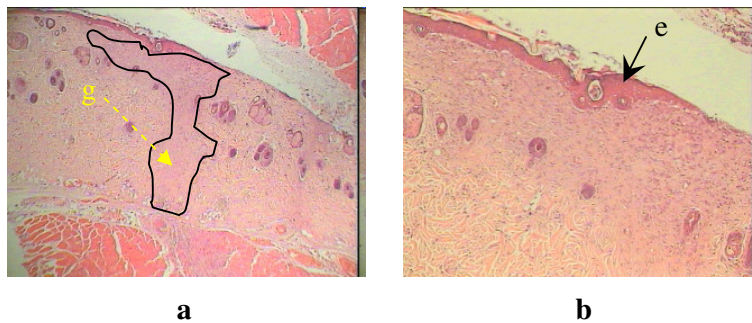


Figure 4.11 Healing of sutured tissue at day 14 (H&E, a-x4, b-x10). Picture **b** is the x10 magnified part of picture **a**. Epidermis is shown by “e”; granulation tissue area is shown by “g”.

Between days 14 and 21, wound progressed as expected. By day 21, a full-thickness layer of granulation tissue, which was measuring $\sim 222092.2\mu\text{m}^2$ and narrowing to a fine scar in the mid- and deep dermis, was observed. The intensity of the scar tissue had decreased. The average epidermal thickness was also decreased to $\sim 37.88\mu\text{m}$. The epidermal hyperplasia decreased and the epidermal thickness reached normal width.

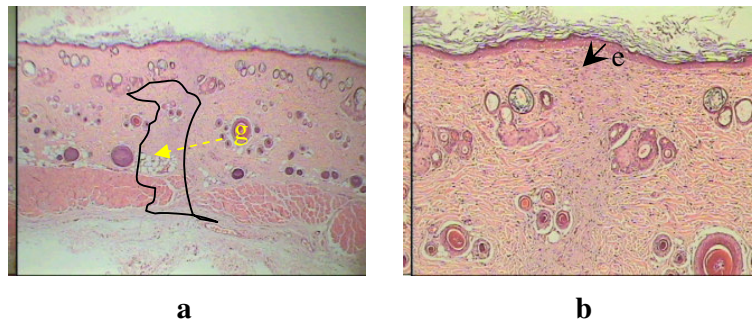


Figure 4.12 Healing of sutured tissue at day 21 (H&E, a-x4, b-x10). Picture **b** is the x10 magnified part of picture **a**. Epidermis is shown by “e”; granulation tissue area is shown by “g”.

In the laser welded tissues, thermal denaturation at the epidermis and above the mid-dermis was indicated by the histological images of wounds taken 24 hour later from the surgery. The denaturation zone disappeared at the deep dermis, while it narrowed in the mid-dermis. The wounds remained open at the epidermis and dermis. The average epidermal thickness of wounds was $\sim 62.46\mu\text{m}$.

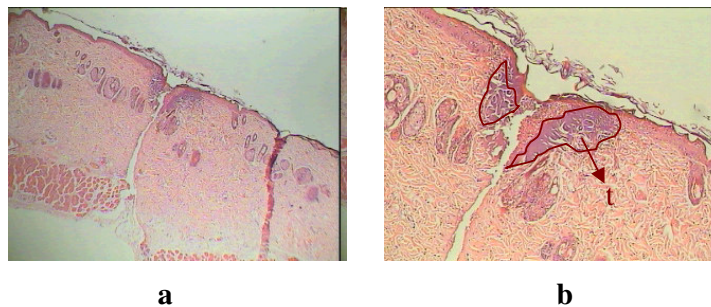


Figure 4.13 Healing of laser welded tissue 24 hours later (H&E, a-x4, b-x10). Thermally denatured zone is shown by “t”. The fluence at a particular point on one spot was 0.76 J/mm^2 .

By day 4, re-epithelialization process was in the stages while the epidermis was beginning to bridge under the denatured, desiccated, and necrotic tissue surface. Complete re-epithelialization was not observed at this stage. Welded wounds were closed at the epidermis, but remained open below epidermis. Migration of the epidermis beneath necrotic tissue and epidermal hyperplasia was observed. Inflammatory cells were present at the wound site. The average epidermal thickness was $\sim 92.81\mu\text{m}$.

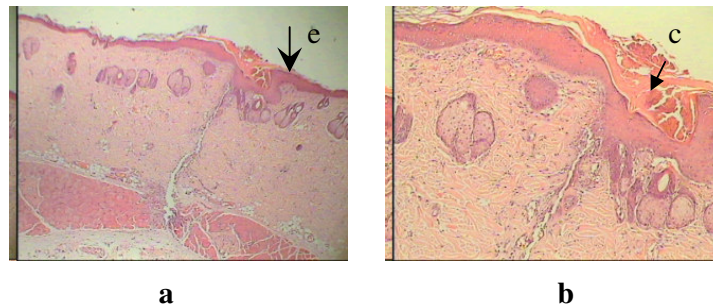


Figure 4.14 Healing of laser welded tissue at day 4 (H&E, a-x4, b-x10). Epidermis is shown by “e”; clot is shown by “c”. The fluence at a particular point on one spot was 0.76 J/mm^2 .

By day 7, re-epithelialization was completed, wounds were fully closed and the average epidermal thickness was $\sim 90.45 \mu\text{m}$. Mid-epidermis and sites around epidermis was marked with fibroblast cells and collagen formation. The average granulation tissue was $\sim 371766.4 \mu\text{m}^2$. Inflammatory cells (Neutrophil leukocyte) had disappeared by day 7.



Figure 4.15 Healing of laser welded tissue at day 7 (H&E, a-x4, b-x10). Epidermis is shown by “e”; granulation tissue area is shown by “g”. The fluence at a particular point on one spot was 0.76 J/mm^2 .

At the 14th day, the epidermal thickness was decreased to $\sim 49.69 \mu\text{m}$. The neo-epidermis had sloughed all of the necrotic tissue in the thermal damage zone above it. Tissue volume was partially replaced with granulation tissue as the epidermal hyperplasia had begun to decrease. The average granulation tissue area was $\sim 288252.8 \mu\text{m}^2$.

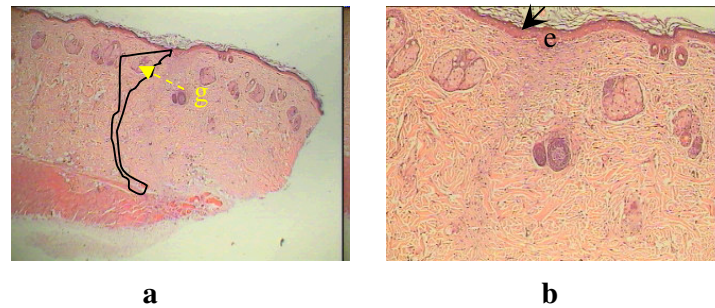


Figure 4.16 Healing of laser welded tissue at day 14 (H&E, a-x4, b-x10). Epidermis is shown by “e”; granulation tissue area is shown by “g”. The fluence at a particular point on one spot was 0.76 J/mm^2 .

From 14 to 21 day, wound healing process progressed routinely. At 21st day, granulation tissue had reached its full-thickness. The average granulation tissue area was $\sim 206982.8 \mu\text{m}^2$. The epidermal thickness was $36.5 \mu\text{m}$.

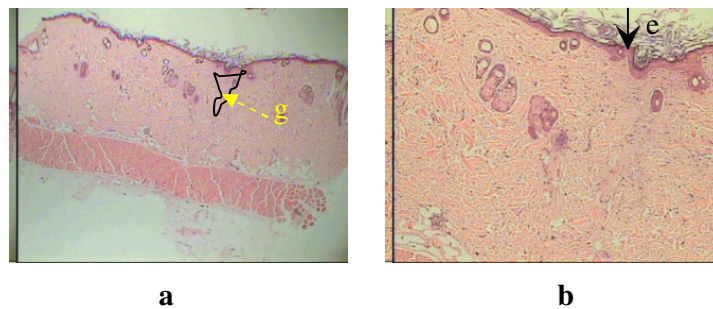


Figure 4.17 Healing of laser welded tissue at day 21 (H&E, a-x4, b-x10). Epidermis is shown by “e”; granulation tissue area is shown by “g”. The fluence at a particular point on one spot was 0.76 J/mm^2 .

Tissue apposition, area of granulation tissue and epidermal thickness were considered as semi-quantitative indicators of wound healing of sutured wounds and laser welds. After 24 hours, analysis of laser and suture skin closure revealed no significant difference in epidermal thickness ($P > 0.25$). The epidermis hypertrophied from its normal thickness of $\sim 45 \mu\text{m}$ to $\sim 113.36 \mu\text{m}$ for sutured wounds and to $\sim 92.81 \mu\text{m}$ for laser welds at day 4. No significant difference between sutured wounds and laser welds was observed ($P > 0.25$). By day 7, as wound healing progressed, the epidermis had begun to decrease in thickness. There was still no significant difference between laser and suture skin closure ($P > 0.25$). By the 14th day, the epidermal thickness of sutured wounds was $\sim 62.04 \pm 7.27 \mu\text{m}$, a value significantly less than the epidermal thickness of laser welded skin measured $\sim 49.69 \pm 6.3 \mu\text{m}$ ($P < 0.05$). However, at day 21, there was again no significant

difference between the epidermal thickness' of the laser and suture groups (Table 4.2, Figure 4.18).

Table 4.2

Average epidermal thickness' of laser welded and sutured groups.

Control Days	Epidermal Thickness of Suture Group (μm)	Epidermal Thickness of Laser Group (μm)	t-test
1.	62.9 ± 19.5	62.5 ± 6.8	0.940514
4.	113.4 ± 21	92.8 ± 27.9	0.186748
7.	87.4 ± 27.1	90.5 ± 26.2	0.804301
14.	62.0 ± 7.3	49.7 ± 6.3	0.010775
21.	37.9 ± 7.4	36.5 ± 0.9	0.668418

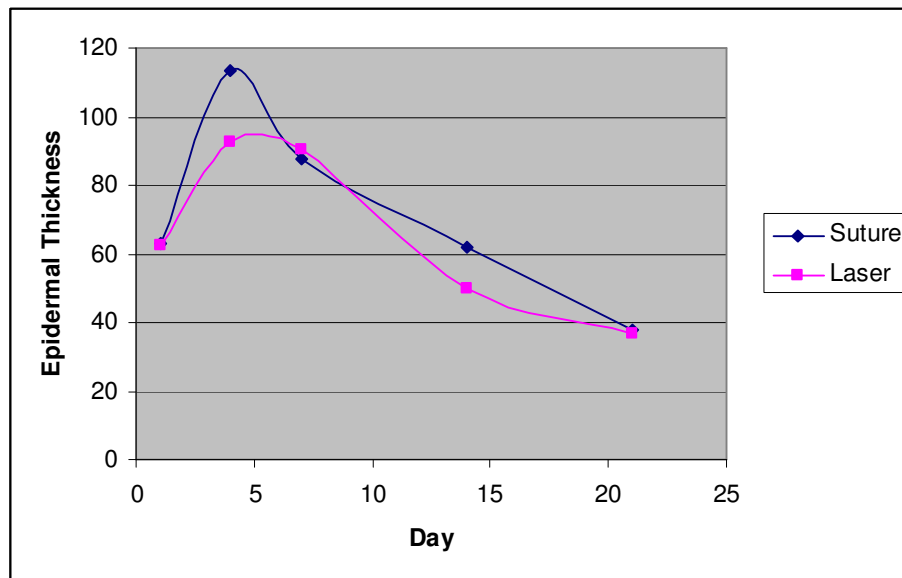


Figure 4.18 Epidermal thickness measurements for tissue closed by either laser or sutures. From day 0 to 21, epidermal thicknesses were increased until day 7, and then it started to decrease.

The area of the granulation tissue for wounds closed with either laser or sutures was measured at the dermis beginning after 7 days. For suture closed wounds, the granulation tissue area was less than that detected in the laser welds. However, no significant difference between the granulation area of laser and suture group was noted. A finer scar

area was present through the full-thickness of the tissue, measuring $\sim 222092.2 \pm 75102.03\mu\text{m}^2$ at the 21st day.

For the laser welds, granulation area in the papillary dermis was more extended compared to that in the mid-dermis and the base of the dermis. Increased granulation in the papillary dermis was due to thermal damage. At 14 and 21 days, the epidermis began to recede in area and sloughed necrotic tissue from the wound site. The granulation area was $\sim 371766,4 \pm 582636,1\mu\text{m}^2$ 24 hours later and the granulation area was $\sim 206982,8 \pm 51178,09\mu\text{m}^2$ at the 21st day (Table 4.3, Figure 4.19).

Table 4.3

Average granulation area of lasered and sutured groups.

Control Days	Granulation Area of Suture Group (μm^2)	Granulation Area of Laser Group (μm^2)	t-test
7.	197190.3 ± 89554.9	371766.4 ± 582636.1	0,504033
14.	259115.2 ± 133087.9	208252.8 ± 241571.7	0,802523
21.	222092.2 ± 75102.03	206982.8 ± 51178.1	0,69354

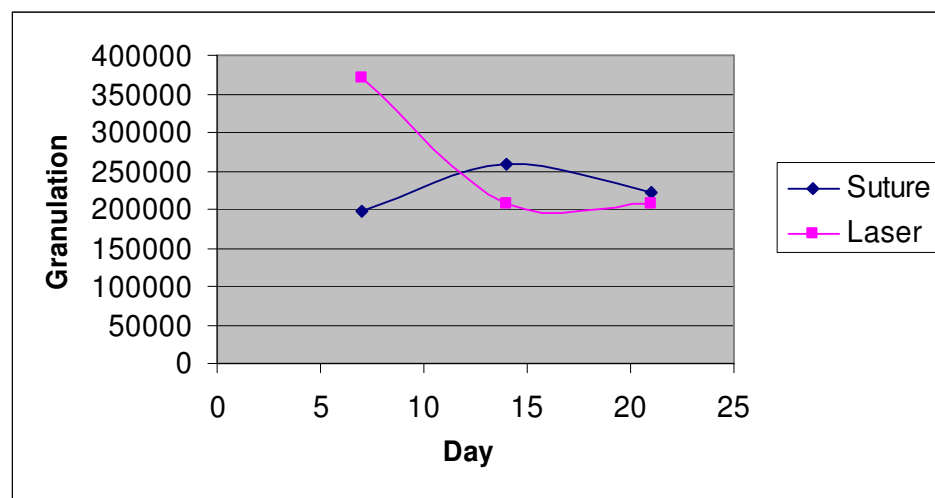


Figure 4.19 Granulation tissue area measurements (μm^2) for tissue closed by either laser or suture.

Lasered and sutured wounds were photographed at the control days. During the early phase of wound healing, the tissue was more uniformly sealed along the entire 1-cm-long wound for the laser welded group. The sutured wounds, use of needles caused a scar line perpendicular to the incision site.

5. DISCUSSION

Lasers have been used for bonding tissues in animal models for more than 30 years. However, laser welding does not find wide areas of applications in the clinical realm due to several major difficulties of the technique. The first hindrance is lack of consistency while the other obstacle is the collateral thermal injury, which is a function of time and temperature. In a series of welding procedures, a desired level of bonding can be obtained while others exhibit very poor bonding [23]. Acceleration of wound healing without thermal damage and an improvement consistency in bonding of tissues by assisting skin closure with a 980-nm diode laser is put forward in the current study.

In this study, tissue welding with 980-nm laser system, which is a novel approach in the laser tissue welding, was performed. Effects of 980-nm diode laser on tissue welding are not studied before. Hence, determination of optimal parameters for further studies was required and a preliminary study was performed for this purpose. Based on the results of preliminary studies, laser welding with 980-nm diode laser was practiced and compared to traditional suture technique. The major advantage of 980-nm diode laser is that the absorption coefficients for water is less than the absorption coefficient of CO₂ laser, which is used in laser soldering study by Simhon and his colleagues [24]. At the wavelength of CO₂ laser, light is very strongly absorbed by water, and water is a large constituent of skin (%65.3)[25]. Most of the CO₂ energy is absorbed in the outermost layers of the tissue, and little of that energy penetrates to the deeper layers, whereas 980-nm laser can penetrate to the deeper layers. Simhon and his colleagues use albumin solder to provide the adherence of the stratum corneum and the dermal layers. In our study, these layers were adhered without albumin solder. In addition to the absorption coefficient for water, the absorption coefficient of 980-nm laser for melanin is less than the absorption coefficients of 780-nm and 815-nm diode lasers, which were used by Decoste, and Capon [26, 10]. Relatively low absorption coefficient of 980-nm diode laser for melanin leads to the penetration of laser energy to deeper layers. Since high absorption coefficients of 780-nm and 815-nm diode lasers for melanin prevents deeper penetration; a transparent adhesive dressing was needed

to appose incisions in Capon's study, while ICG was used to provide good penetration in the tissue in Decoste's study.

Estimation of proper optimal laser parameters enabled us to obtain smaller thermal denaturation zone measured during the *in vivo* studies compared to the preliminary studies. The parameters, which were used in the first experiment of the preliminary study lead to significant thermal damage on the most of the incisions. The energy (48 J) used in the third wound seemed to give best result. Estimation of welding parameters for the second experiment of preliminary study was guided by the results of the first experiment and additional literature surveys. Relatively better results (i.e. less thermal damage and faster wound healing) were obtained in the second experiment compared to the first one.

In the second experiment of the preliminary study, laser welding by various parameters did not yield significantly different results. Thus, the optical irradiance (0.76 J/mm^2) of the wounds number 1-3 of the first animal was chosen. Providing less exposure of laser to the skin tissue, leading less thermal damage was the main reason of this preference.

Histological analysis revealed that there were no significant differences between wound healing of laser welded and sutured tissues when the welded and suture techniques were compared. Minimal thermal injury was seen in the laser welded tissue only 24 hours later. Carbonaceous substances were not seen on the epidermis. Better apposition was noticed in laser group, when compared with sutured cuts. No significant difference between the epidermal thicknesses of two groups was observed except for 14th day. At day 14, laser welded tissues had thinner epidermis than sutured tissues. This indicated that, laser welded tissues returned their normal thicknesses faster after the thermal effect decreased.

In both groups, similar foreign body reaction was observed due to hair follicles that burst during the procedure of skin cutting, laser welding and suturing. Mild inflammation was observed in the very early days of wound healing in both groups. More inflammation

in the sutured group compared to laser welds was expected. The reason for lack of such visible inflammation in sutured group may be attributed to short slice lengths, which makes needle holes totally invisible.

Another semi-quantitative evaluation indicator is the area of granulation tissue. No gross difference between laser welded and sutured tissues in granulation tissue area is parallel to a previous study performed by Fried [15]. In laser groups, the granulation zone was broad near epidermis, and disappeared in the mid-dermis as a result of the decreased thermal effect on the mid-dermis. On the other hand, in sutured group, granulation occurred in the papillary dermis than narrowed through the mid-dermis and expanded on the base of the dermis.

The major achievement of this study is that the ability of 980-nm laser diode system to close incisions with minimal thermal damage has been shown. Also Laser welding with 980-nm laser system has been introduced as a potential alternative technique to suturing. One limitation in our study is lack of precise temperature control of the tissue during welding by means of thermocouples [14]. Beside thermocouples, a translational stage should be used to provide uniform exposure of laser radiation [15]. Further refinement and optimization of the welding parameters will be necessary to produce full-thickness welds, less thermal damage in the epidermis and upper-dermis.

6. CONCLUSION

The main goal of this study was to examine effects of 980-nm diode laser on skin welding and to compare laser welding with suture technique. Thermal damage, a major side-effect of tissue-bonding, was minimized by estimation of optimal parameters. Reliable histological wound healing semi-quantitative indicators and macroscopic evaluations were used for comparison of laser and suture groups. These analyzing methods showed that there were no significant differences in wound healing procedure of laser welded and sutured tissues. At the end of the experiments, the rats did not show any abnormality on their health, behavior and nutrition manner.

According to the results and discussion of our studies, it is concluded that tissue welding with 980-nm diode laser can be a good candidate for tissue welding applications.

In future studies

- Different laser parameters can be used in tissue welding.
- In addition to 980-nm diode laser, 809-nm diode laser can be used.
- Number of samples used in experiments can be increased.
- Laser tissue welding can be performed with an albumin solder.
- Tensile strength measurements can be done to compare tensile strengths of laser and suture groups.

APPENDIX

This section includes the photographs of the incisions closed by suture and laser welding. Photographs are listed in order of the control days; 1, 4, 7, 4 and 21. Sutured incisions are denoted by “S “, laser welded incisions are denoted by “L “.

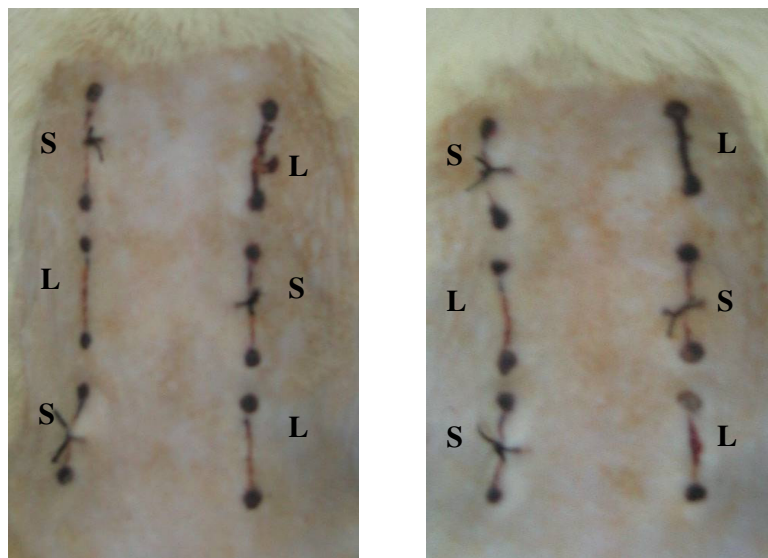


Figure 1. Images of wounds on the 1st day

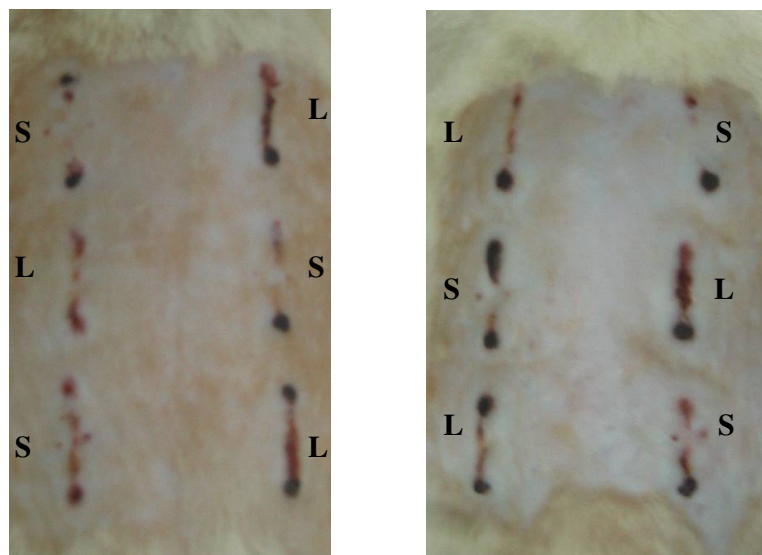


Figure 2. Images of wounds on the 4th day

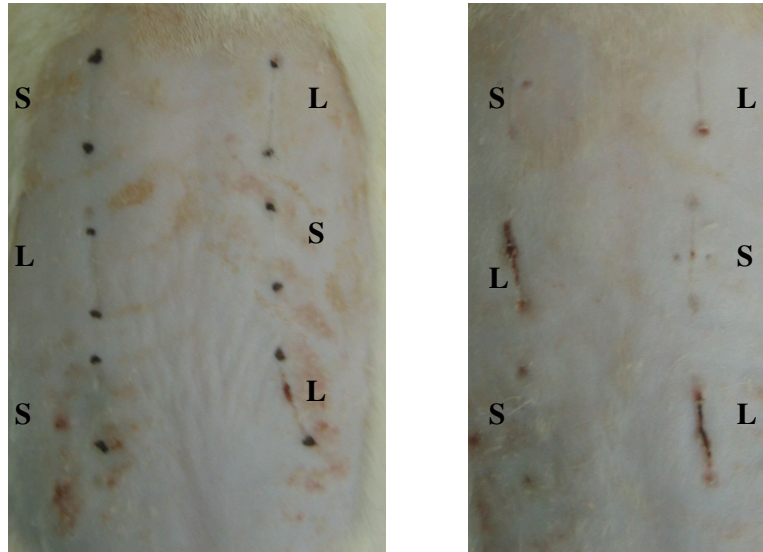


Figure 3. Images of wounds on the 7th day

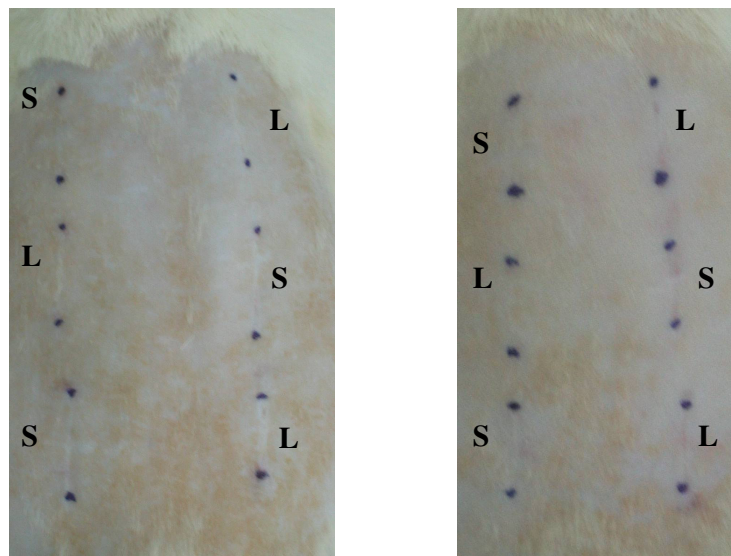


Figure 4. Images of wounds on the 14th day

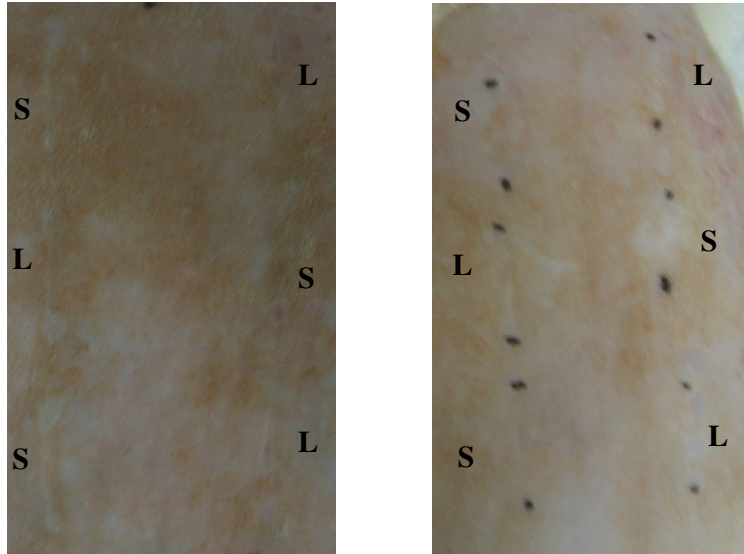


Figure 5. Images of wounds on the 21th day

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